

## CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients

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### Supplementary Table 1 Oligonucleotides for generating sgRNA expression vectors

Primers No.	Primer Sequences (5' to 3')
hPD1sg1up	ACCGCGTGA CTTCACATGAGCG
hPD1sg1down	AAACCGCTCATGTGGAAGTCACG
hPD1sg2for	ACCGCAGTTGTGTGACACGGAAG
hPD1sg2down	AAACCTTCCGTGT CACACA ACTG
hPD1sg3for	ACCGACAGCGGCACCTACCTCTG
hPD1sg3down	AAACCAGAGGTAGGTGCCGCTGT
hPD1sg4for	ACCGGGCCCTGACCACGCTCATG
hPD1sg4down	AAACCATGAGCGTGGTCAGGGCC

**Supplementary Table 2** Sequences of primers for dual sgRNA plasmids produce

Primers No.	Primer Sequences (5' to 3')
hPD1-Psg1	ATGCGTCTCAACCGCGTGACTTCCACATGAGCGGTTTTAGAGCTAGAAATAGCAAG
hPD1-Psg2	ATGCGTCTCGAAACCTTCCGTGTACACAACCTGCGGTGTTTCGTCTTCCACAAG
hPD1-Psg3	ATGCGTCTCAACCGACAGCGGCACCTACCTCTGGTTTTAGAGCTAGAAATAGCAAG
hPD1-Psg4	ATGCGTCTCGAAACCATGAGCGTGGTCAGGGCCCGGTGTTTCGTCTTCCACAAG

**Supplementary Table 3** Sequences of primers for PCR amplification of targeting sites

Primers No.	Primer Sequences (5' to 3')	Amplicon (bp)
hPD1testF	GGACAACGCCACCTTCACCTGC	390
hPD1testR	CTACGACCTGGAGCTCCTGAT	

**Supplementle Figure 1** Sequences of modified hPD-1 loci detected in the HeLa cellssequencing

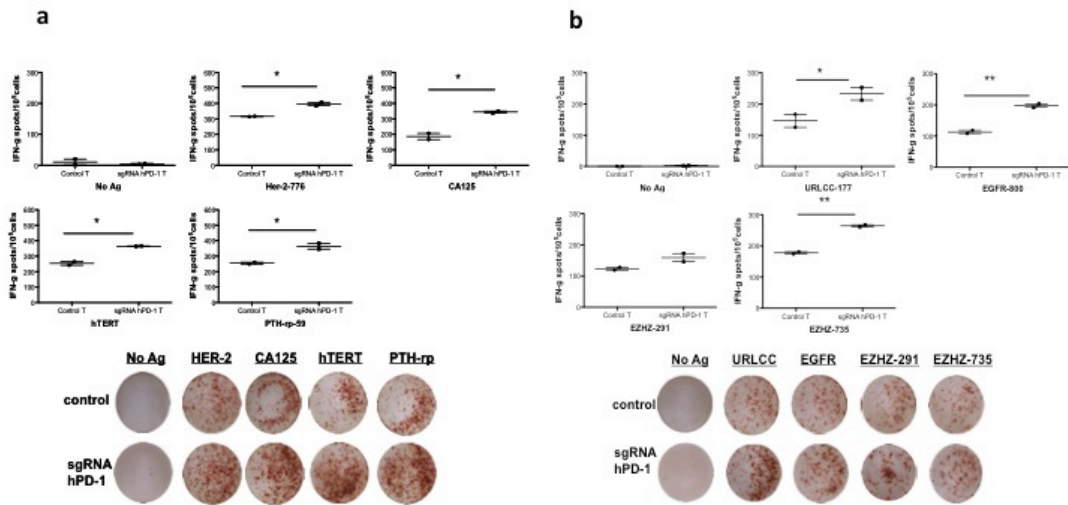
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hPD1
sg1  TGTCACACAACCTGCCAACGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCGGGCAATGAC (WT)
      TGTCACACAACCTGCCAACGGCGGTGACTTCCACAT : AGCGTGGTCAGGGCCCGGGCAATGAC (-1, 1/21)
      TGTCACACAACCTGCCAACGGCGGTGACTTCCACAT : GCGTGGTCAGGGCCCGGGCAATGAC (-2, 2/21)
      TGTCACACAACCTGCCAACGGCGGTGACTTCCACA : GCGTGGTCAGGGCCCGGGCAATGAC (-3, 1/21)
      TGTCACACAACCTGCCAACGGCGGTGACTTCCAC : GCGTGGTCAGGGCCCGGGCAATGAC (-4, 1/21)
      TGTCACACAACCTGCCAACGGCGGTGACTTCCAC : GCGTGGTCAGGGCCCGGGCAATGAC (-8, 1/21)
      TGTCACACAACCTGCCAACGGCGG : TGAGCGTGGTCAGGGCCCGGGCAATGAC (-11, 1/21)
      TGTCACACAACCTGCCAACGGCGGTGACTTCCACATGAGCGTGGTCAGGGCCCGGGCAATGAC (+1, 6/21)
      a
sg2  CAGCCCGGCCAGGACTGCCGCTTCCGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGA (WT)
      CAGCCCGGCCAGGACTGCCGCT : GTCACACAACCTGCCAACGGCGGTGACTTCCACATGA (-5, 1/19)
      CAGCCCGGCCAGGACTGCCGC : GTCACACAACCTGCCAACGGCGGTGACTTCCACATGA (-6, 1/19)
      CAGCCCGGCCAGGACTGCCGCTT : ACAACTGCCAACGGCGGTGACTTCCACATGA (-9, 1/19)
      CAGCCCGGCC : CCGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGA (-13, 1/19)
      CAGCCCGGC : CGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGA (-15, 1/19)
      CAGC : ACACAACCTGCCAACGGCGGTGACTTCCACATGA (-26, 1/19)
      CAGCCCGGCCAGGA : CGGCGGTGACTTCCACATGA (-30, 1/19)
      CAGCCCGGCCAGGACTGCCGCTTCCGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGA (+1, 3/19)
      c
      CAGCCCGGCCAGGACTGCCGCTTCCGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGA (+51, 2/19)
      bcagaaagctgcagggtttgtccataggtatacgtgtgcatggtggtttgc
sg1+2 CTGCGCCTTCCGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGAGCGTGGTCAGGGCC (WT)
      CTGCCGCTTC : AGCGTGGTCAGGGCC (-39, 1/21)
      CTGC : GCGTGGTCAGGGCC (-46, +1, 1/21)
      CTGCCGCTT : AGCGTGGTCAGGGCC (-40, +1, 2/21)
      CTGCCGCTT : gAGCGTGGTCAGGGCC (-40, +2, 1/21)
      : CGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGA : AGCGTGGTCAGGGCC (-22, -9, 1/21)
      CTGCCGC : CGTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATGA : GTGGTCAGGGCC (-3, -2, +1, 1/21)
      CTGCC : GTGTGCACACAACCTGCCAACGGCGGTGACTTCCACATG : tggg AGGGCC (-6, -9, +4, 1/21)
      CTGCC : GTGTGCACACAACCTGCCAACGGCGGTGACTTCCAC : GCGTGGTCAGGGCC (-6, -4, +1, 1/21)
      : acccggg : CGGCGGTGACTTCCACATGAGCGTGGTCAGGGCC (-25, +7, +1, 1/21)
      a

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The PAM sequences are underlined and highlighted in green; the targeting sequences in red; the mutations in blue, lower case; deletions (-), insertions (+). N/N indicates positive colonies out of total sequenced.

**Supplemente Figure 2** The enhanced IFN-gamma production by the Cas9-mediated disruption of PD-1 in primary T cells of cancer patients stimulated by tumor associated antigens



sgRNA hPD-1:Cas9 modified primary T cells and control T cells from cancer patients were cultured in IL-2 after electroporation for 7 d and stimulated by peptides pulsed autologous DCs for 20 h. The IFN- $\gamma$  secreting cells was evaluated by Elispot. All values shown are mean  $\pm$  SD of triplicate or duplicate measurements and have been repeated 3 times with similar results. \* $p < 0.05$ ; \*\* $p < 0.005$ .